RISK ASSESSMENT: SELECTED ORGAN AND COMPOUND-RELATED ISSUES

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Mechanistic Data and Risk Assessment of Selected Toxic End Points of the Thyroid Gland*

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ABSTRACT

Many goitrogenic xenobiotics that increase the incidence of thyroid tumors in rodents exert a direct effect on the thyroid gland to disrupt one of several possible steps in the biosynthesis, secretion, and metabolism of thyroid hormones. This includes (a) inhibition of the iodine trapping mechanism, (b) blockage of organic binding of iodine and coupling of iodothyronines to form thyroxine (T_4) and triiodothyronine (T_3), and (c) inhibition of thyroid hormone secretion by an effect on proteolysis of active hormone from the colloid. Another large group of goitrogenic chemicals disrupts thyroid hormone economy by increasing the peripheral metabolism of thyroid hormones through an induction of hepatic microsomal enzymes. This group includes central nervous system–acting drugs, calcium channel blockers, steroids, retinoids, chlorinated hydrocarbons, polyhalogenated biphenyls, and enzyme inducers. Thyroid hormone economy also can be disrupted by xenobiotics that inhibit the 5′-monodeiodinase that converts T_4 in peripheral sites to biologically active T_3. Inhibition of this enzyme by FD&C Red No. 3 lowers circulating T_3 levels, which results in a compensatory increased secretion of thyroid stimulating hormone (TSH), follicular cell hypertrophy and hyperplasia, and an increased incidence of follicular cell tumors in 2-yr or lifetime studies in rats. Physiologic perturbations alone, such as the feeding of an iodine-deficient diet, partial thyroidectomy, natural goitrogens in certain foods, and transplantation of TSH-secreting pituitary tumors in rodents also can disrupt thyroid hormone economy and, if sustained, increase the development of thyroid tumors in rats. A consistent finding with all of these goitrogens, be they either physiologic perturbations or xenobiotics, is the chronic hypersecretion of TSH, which places the rodent thyroid gland at greater risk to develop tumors through a secondary (indirect) mechanism of thyroid oncogenesis associated with hormonal imbalances.

Keywords. Thyroid tumors; hepatic microsomal enzyme induction; inhibitors of 5′-monodeiodinase; secondary (indirect) mechanisms of thyroid oncogenesis; hormonal imbalances; FD&C Red No. 3 (erythrosine); thyroperoxidase inhibition; thyroid stimulating hormone (TSH); thyroxine (T_4); triiodothyronine (T_3); reverse triiodothyronine (rT_3)

INTRODUCTION

Long-term perturbations of the pituitary–thyroid axis by various xenobiotics or physiologic alterations (e.g., iodine deficiency, partial thyroidectomy, natural goitrogens in food) are more likely to predispose the laboratory rat to a higher incidence of proliferative lesions (e.g., hyperplasia and adenomas of follicular cells) in response to chronic thyroid stimulating hormone (TSH) stimulation than in the human thyroid (3, 11). This is particularly true in the male rat, which has higher circulating levels of TSH than females. The greater sensitivity of the rodent thyroid to derangement by drugs, chemicals, and physiologic perturbations also is related to the shorter plasma half-life of thyroxine (T_4) than in humans due to the considerable differences between species in the transport proteins for thyroid hormones (12).

The plasma T_4 half-life in rats is considerably shorter (12–24 hr) than in humans (5–9 days). In human beings and monkeys, circulating T_4 is bound primarily to thyroxine-binding globulin (TBG), but this high-affinity binding protein is not present in rodents, birds, amphibians, or fish (Table I).

The binding affinity of TBG for T_4 is approximately 1,000 times higher than for prealbumin. The percentage of unbound active T_4 is lower in species with high levels of TBG than in animals in which T_4 binding is limited to albumin and prealbumin. Therefore, a rat without a functional thyroid requires about 10 times more T_4 (20 μg/kg body weight) for full substitution than an adult human (2.2 μg/kg body weight). Triiodothyronine (T_3) is transported bound to TBG and albumin in humans, monkey, and dog but only to albumin in mouse, rat, and chicken (Table II). In general, T_3 is bound less avidly to transport proteins than T_4, resulting in a faster turnover and shorter plasma half-life in most species. These differences in plasma half-life of thyroid hormones and binding to transport proteins between rats and humans may be one factor in the greater sensitivity of the rat

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TABLE I.—T_4 binding to serum proteins in selected vertebrate species.

Species	T_4-binding globulin	Postalbumin	Albumin	Prealbumin
Human	++	—	++	+
Monkey	++	—	++	+
Dog	+	—	++	—
Mouse	—	++	++	—
Rat	—	+	++	+
Chicken	—	—	++	—

Codes: + or ++ = degree of T_4 binding to serum proteins; — = absence of binding of T_4 to serum protein.

From K.-D. Döhler, C. C. Wong, and A. vz Muhlen, 1979, The rat as model for the study of drug effects on thyroid function: Consideration of methodological problems, *Pharmacol. Ther.* 5: 305.

thyroid to develop hyperplastic and/or neoplastic nodules in response to chronic TSH stimulation.

Review of the *U.S. Physicians' Desk Reference* (1994) reveals a number of marketed drugs that resulted in a thyroid tumorigenic response when tested at high concentrations in rodents, primarily in rats. A broad spectrum of product classes are represented including antibiotics, calcium channel blockers, antidepressants, and hypolipidemic agents, among others (Fig. 1). Amiodarone (an antiarrhythmic drug) and iodinated glycerol (an expectorant) are highly iodinated molecules that disrupt thyroid hormone economy by mechanisms similar to the food color, FD&C Red No. 3 (Fig. 2).

MECHANISMS OF TOXICITY OF THYROID FOLLICULAR CELLS

Goitrogenic Chemicals and Thyroid Tumors in Rodents

Numerous studies have reported that chronic treatment of rodents with goitrogenic compounds results in the development of follicular cell adenomas. Thiouracil and its derivatives have this effect in rats (25) and mice (24). This phenomenon also has been observed in rats that consumed brassica seeds (7), erythrosine (FD&C Red No. 3) (2, 3), sulfonamides (33), and many other compounds (16, 30).

The pathogenetic mechanism of this phenomenon has been understood for some time and is widely accepted (15). These goitrogenic agents either directly interfere with thyroid hormone synthesis or secretion in the thyroid gland, increase thyroid hormone excretion into the bile, or disrupt the peripheral conversion of T_4 to T_3. The ensuing decrease in circulating thyroid hormone levels results in a compensatory increased secretion of pituitary TSH. The receptor-mediated TSH stimulation of the thyroid gland leads to proliferative changes of follicular cells that include hypertrophy, hyperplasia, and, ultimately, neoplasia in rodents. In the multistage model of carcinogenesis, proliferative lesions often begin as hyperplasia, may proceed to the development of benign tumors (adenoma), and infrequently develop into a malignant tumor. Although these lesions usually are classified as discrete entities, it is important to emphasize that they represent a morphologic continuum (Fig. 3) with imprecise criteria to separate borderline proliferative lesions.

Excessive secretion of TSH alone (i.e., in the absence of any chemical exposure) also has been reported to produce a high incidence of thyroid tumors in rodents. This has been observed in rats fed an iodine-deficient diet (1) and in mice that received TSH-secreting pituitary tumor transplants (15). The pathogenetic mechanism of thyroid

TABLE II.—Triiodothyronine (T_3) binding to serum proteins in selected vertebrate species.

Species	T_3-binding globulin	Postalbumin	Albumin	Prealbumin
Human	+	—	+	—
Monkey	+	—	+	—
Dog	+	—	+	—
Mouse	—	+	+	—
Rat	—	—	+	—
Chicken	—	—	+	—

Codes: + or ++ = degree of T_3 binding to serum proteins; — = absence of binding of T_3 to serum protein.

From K.-D. Döhler, C. C. Wong, and A. vz Muhlen, 1979, The rat as model for the study of drug effects on thyroid function: Consideration of methodological problems, *Pharmacol. Ther.* 5: 305.

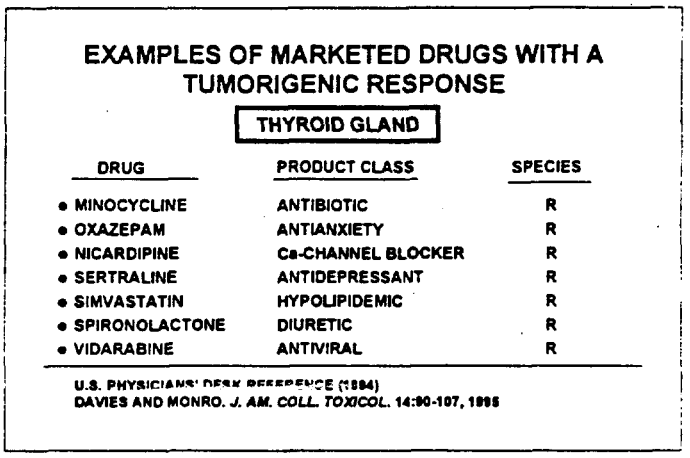

FIG. 1.—Examples of marketed drugs with a tumorigenic response in the thyroid gland of rats. (Modified from T. S. Davis and A. Monro, 1995, Marketed human pharmaceuticals reported to be tumorigenic in rodents, *J. Am. Coll. Toxicol.* 14: 90.)

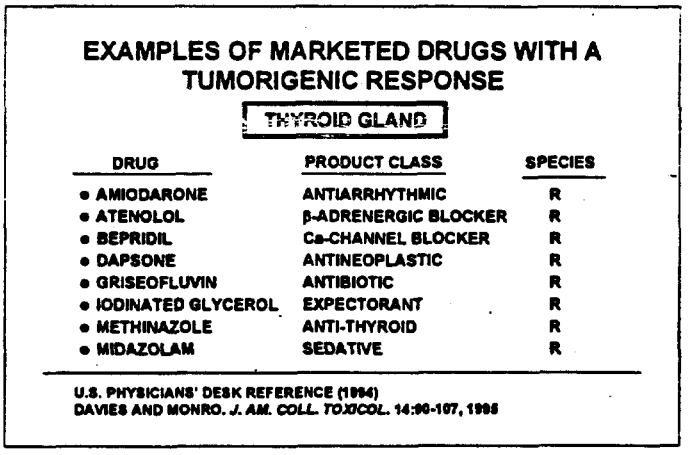

FIG. 2.—Marketed drugs with a thyroid tumorigenic response. (Modified from T. S. Davis and A. Monro, 1995, Marketed human pharmaceuticals reported to be tumorigenic in rodents, *J. Am. Coll. Toxicol.* 14: 90.)

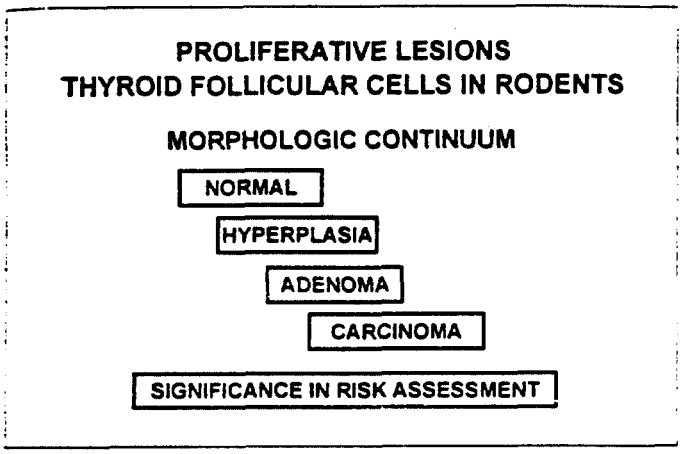

FIG. 3.—Proliferative changes in the multistage model of carcinogenesis represent a morphologic continuum with an overlap in morphologic features.

follicular cell tumor development in rodents involves a sustained excessive stimulation of the thyroid gland by TSH. In addition, iodine deficiency is an important promoter of the development of thyroid tumors in rodents induced by intravenous injection of *N*-methyl-*N*-nitrosurea (Fig. 4) (28).

Effects of Xenobiotic Chemicals on Peripheral Metabolism of Thyroid Hormones

Inhibition of 5'-Deiodinase. FD&C Red No. 3 (erythrosine) is an example of a well-characterized xenobiotic that results in perturbations of thyroid function in rodents and in long-term studies is associated with an increased incidence of benign thyroid tumors. Red No. 3 is a widely used color additive in foods, cosmetics, and pharmaceuticals. A chronic toxicity/carcinogenicity study revealed that male Sprague-Dawley rats fed a 4% dietary concentration of Red No. 3 beginning *in utero* and extending over their lifetime (30 mo) developed a 22% incidence of well-differentiated thyroid adenomas derived from follicular cells compared to 1.5% in control rats and a historical incidence of 1.8% for this strain (2).

The thyroid adenomas were well demarcated from the normal gland by a thin layer of fibrous connective tissue, and adjacent follicles were slightly compressed. The neoplastic cells were more hyperchromatic than the surrounding normal follicular cells and either formed variably sized colloid-containing follicles, lined large cystic spaces, or formed solid sheets. Some neoplastic cells formed papillary structures extending into cystic spaces. There was no evidence of vascular invasion. In addition, male rats developed a 23% incidence of follicular cystic hyperplasia. These focal lesions were formed by the coalescence of adjacent colloid-distended follicles. The follicular wall (or remnants) was lined by 1 or 2 layers of low cuboidal epithelium with hyperchromatic nuclei that occasionally formed papillary projections. There was not a significant increase in follicular cell adenomas in the lower dose groups of male rats or an increase in malignant thyroid follicular cell tumors. Female rats fed similar amounts of the color did not develop a significant increase in either benign or malignant thyroid tumors. Feeding of the color at the high dose (4%) level provided

PROMOTING EFFECT OF IODINE DEFICIENCY (ID) ON THYROID CARCINOGENESIS INDUCED BY N-NITROSOMETHYLUREA (NMU) AT 33 WEEKS

GROUP	THYROID WEIGHT ($\bar{x}$ mg ± SD)	DIFFUSE FC HYPERPLASIA (%)	FOLLICULAR CELL (FC) ADENOMA (%)	FOLLICULAR CELL (FC) CARCINOMA (%)	TOTAL FOCAL FC LESIONS (no./cm²)
NMU + ID DIET	632 ± 208*	100x	100xx	100xxxx	11 ± 6
NMU + IODINE ADEQUATE (IA) DIET	46 ± 13**	0	70xxx	10	14 ± 14
NMU + CONTROL DIET	29 ± 4***	0	50	0	17 ± 20
ID DIET	109 ± 12****	100x	0	0	0.4 ± 0.8
IODINE ADEQUATE DIET	40 ± 16	0	0	0	0
CONTROL DIET	36 ± 4	0	0	0	0

* P<0.01 vs. GROUPS 2, 3, 4, 5, or 6
** P<0.05 vs. GROUPS 3 or 4
*** P<0.01 vs. GROUP 4
**** P<0.01 vs. GROUPS 5 or 6

x P<0.001 vs. GROUPS 2, 3, 5 or 6
xx P<0.001 vs. GROUPS 4, 5, or 6
xxx P<0.01 vs. GROUPS 4, 5, or 6
xxxx P<0.001 vs. GROUPS 2, 3, 4, 5, or 6

CONTROL DIET = STERILIZABLE WAYNE BLOX DIET; IODINE ADEQUATE = ID (REMINGTON) DIET + 0.01 gm/kg K IODATE.
NMU 41 mg/kg IV AT 6 WK OF AGE (2 WK BEFORE DIETS STARTED).

OHSHIMA AND WARD. *J. NATL. CANCER INST. 73* (1984): 289-296;

FIG. 4.—Data demonstrating the potent promoting effects of iodine deficiency in rats administered a single intravenous dose of a known initiator (*N*-nitrosomethylurea) of thyroid neoplasms. (Modified from M. Ohshima and J. M. Ward, 1984, Promotion of N-methyl-N-nitrosurea-induced thyroid tumors by iodine deficiency in F344/NCr rats, *J. Natl. Cancer Inst.* 73: 289.)

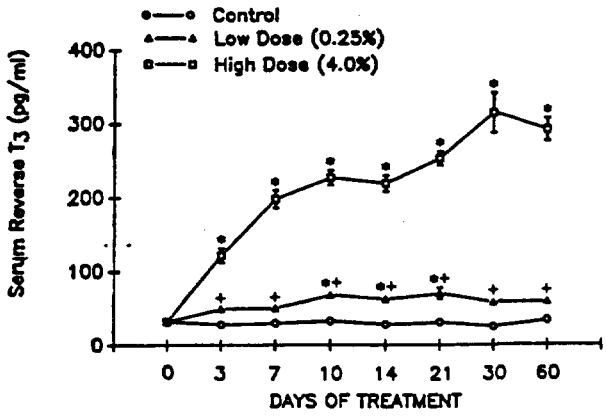

FIG. 5.—Rapid and significant increase in serum reverse triiodothyronine (rT_3) levels in Sprague-Dawley rats (N = 20/group and interval) administered a high (4%) and low (0.25%) dose of FD&C Red No. 3. The significant increase in rT_3 was detected at the initial interval of 3 days and persisted during the 60-day experiment in the high-dose group. (Courtesy of the Certified Color Manufacturers Association, Inc., and Drs. L. E. Braverman and W. J. DeVito, University of Massachusetts Medical School.)

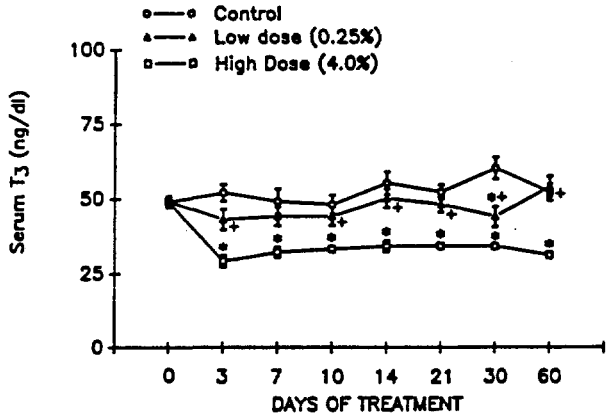

FIG. 6.—Changes in serum T_3 following administration of a high (4%) and low (0.25%) dose of FD&C Red No. 3 in the diet to Sprague-Dawley rats. (Courtesy of the Certified Color Manufacturers Association, Inc., and Drs. L. E. Braverman and W. J. DeVito, University of Massachusetts Medical School.)

male rats with 2,464 mg/kg of Red No. 3 daily; by comparison, human consumption in the United States is estimated to be 0.023 mg/kg/day.

The results of mechanistic studies have suggested that a primary (direct) action of FD&C Red No. 3 on the thyroid is unlikely due to (a) failure of the color (^{14}C-labeled) to accumulate in the gland, (b) negative genotoxicity and mutagenicity assays, (c) lack of an oncogenic response in mice and gerbils, (d) a failure to result in thyroid tumor development at dietary concentrations of 1.0% or less in male and female rats (3), and (e) a lack of increased tumor development in other organs. Investigations with radiolabeled compound have demonstrated that the color does not accumulate in the thyroid glands of rats following the feeding of either 0.5 or 4.0% FD&C Red No. 3 for 1 wk prior to the oral dose of ^{14}C-labeled material.

Subsequent mechanistic investigations included a 60-day study in male Sprague-Dawley rats fed either 4% (high dose) or 0.25% (low dose) FD&C Red No. 3 compared to controls in order to determine the effects of the color on thyroid hormone economy and morphometric changes in follicular cells. The experimental design of the study was to terminate groups of rats (n = 20/interval and dose) fed Red No. 3 and controls after 0, 3, 7, 10, 14, 21, 30, and 60 days.

A consistent effect of Red No. 3 on thyroid hormone economy was the striking increase in serum reverse T_3 (Fig. 5). In the high-dose rats, reverse T_3 was increased at all intervals compared to controls and at 10, 14, and 21 days in the low-dose group. The mechanisms responsible for the increased serum reverse T_3 appear to be, first, substrate (T_4) accumulation due to 5′-deiodinase inhibition with subsequent conversion to reverse T_3 rather than active T_3 and, second, reverse T_3 accumulation due to 5′-deiodinase inhibition resulting in an inability to degrade reverse T_3 further to diiodothyronine (T_2).

Serum triiodothyronine (T_3) was decreased significantly at all intervals in rats of the high-dose group compared to interval controls (Fig. 6). The mechanism responsible for the reduced serum T_3 following feeding of Red No. 3 was decreased monodeiodination of T_4 due to an inhibition of the 5′-deiodinase by the color.

Serum TSH was increased significantly at all intervals in rats of the high-dose (4%) group compared to controls. Rats fed 0.25% Red No. 3 had increased serum TSH only at days 21, 30, and 60 (Fig. 7). The mechanism responsible for the increased serum TSH following ingestion of Red No. 3 was a compensatory response by the pituitary gland to the low circulating levels of T_3 that resulted from an inhibition of the 5′-deiodinase.

Serum T_4 also was increased significantly at all intervals in rats fed 4% Red No. 3 compared to controls (Fig. 8). The mechanism responsible for the increased serum

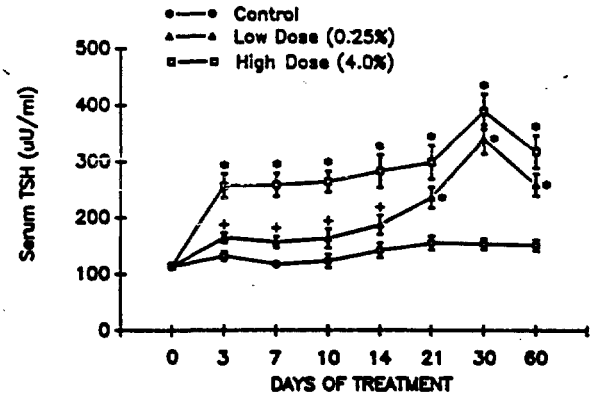

FIG. 7.—Rapid significant increase in serum TSH in Sprague-Dawley rats (N = 20 rats/group and interval) administered a mild goitrogen (FD&C Red No. 3) in the feed. Blood for the TSH assay was collected terminally from the abdominal aorta. The significant increase in serum TSH persisted from day 3 to day 60 of the experiment in the high-dose (4%) group. (Courtesy of Drs. L. E. Braverman and W. J. DeVito, University of Massachusetts Medical School, and Certified Color Manufacturers Association, Inc.)

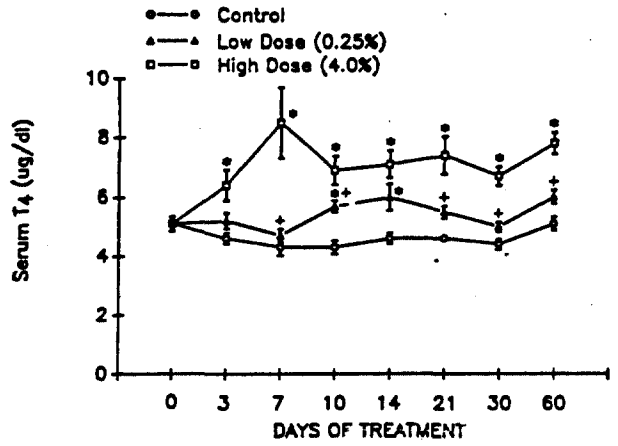

FIG. 8.—Changes in serum T_4 following administration of a high (4%) and low (0.25%) dose of FD&C Red No. 3 in the diet to Sprague-Dawley rats. (Courtesy of the Certified Color Manufacturers Association, Inc., and Drs. L. E. Braverman and W. J. DeVito, University of Massachusetts Medical School.)

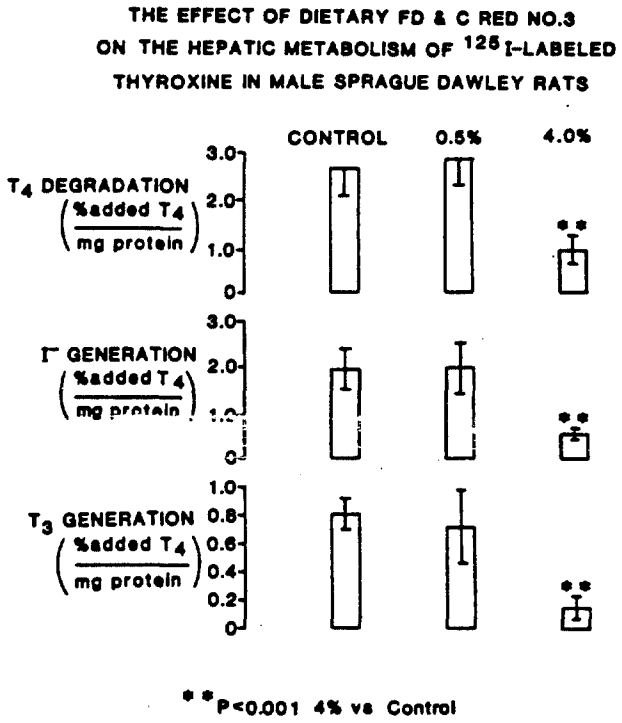

FIG. 9.—Effects of dietary FD&C Red No. 3 on the hepatic metabolism of ^{125}I-labeled thyroxine in male Sprague-Dawley rats. (Courtesy of the Certified Color Manufacturers Association, Inc., and the late Sidney H. Ingbar, M.D.)

T_4 was, first, accumulation due to an inability to monodeiodinate T_4 to T_3 in the liver and kidney from the inhibition of 5'-deiodinase by the color and, second, TSH stimulation of increased T_4 production by the thyroid gland.

^{125}I-labeled T_4 metabolism was significantly altered in liver homogenates prepared from rats fed 4% FD&C Red No. 3. Degradation of labeled T_4 was decreased to approximately 40% of values in control homogenates (Fig. 9). This was associated with a 75% decrease in percentage of generation of ^{125}I and an approximately 80% decrease in percentage of generation of ^{125}I-labeled T_3 from radiolabeled T_4 substrate. These mechanistic investigations suggested that the color results in a perturbation of thyroid hormone economy in rodents by inhibiting the 5'-deiodinase in the liver (Fig. 10), resulting in long-term stimulation of follicular cells by TSH, which over their lifetime predisposed to an increased incidence of thyroid tumors (2, 3). The color was negative in standard genotoxic and mutagenic assays and did not increase the incidence of tumors in other organs.

Morphometric evaluation was performed on thyroid glands from all rats at each interval during the 60-day study. Four levels of thyroid were evaluated with 25 measurements from each rat using a Zeiss interactive digital analysis system at a magnification of ×450. The direct measurements included diameter of thyroid follicles, area of follicular colloid, and height of follicular cells. Thyroid follicular diameter was decreased significantly in both low- and high-dose groups at 3, 7, 10, and 14 days compared to interval controls. The area of follicular colloid generally reflected the decrease in thyroid follicular diameter and was decreased significantly at days 3 and 10 in high-dose rats and days 7 and 10 in the low-dose group compared to interval controls. These reductions in thyroid follicular diameter and colloid area were consistent with morphologic changes expected from an increased serum TSH concentration.

Thyroid follicular height was increased significantly only after feeding FD&C Red No. 3 for 60 days in both the high- and low-dose groups compared to interval controls. The absence of morphometric evidence of follicular cell hypertrophy at the earlier intervals was consistent with the modest increase (+5.8%) in thyroid gland : body weight ratio after this relatively short exposure to the color. The lack of follicular cell hypertrophy at the earlier intervals of feeding Red No. 3 in rats with severalfold

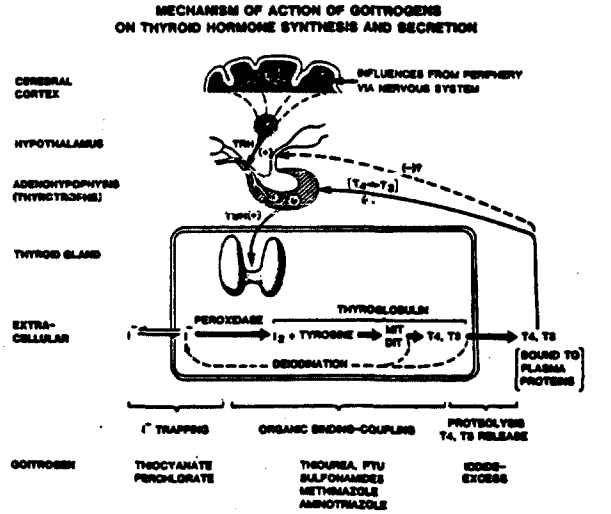

FIG. 10.—Mechanism of action of goitrogens on thyroid hormone synthesis and section. (From C. C. Capen and S. L. Martin, with permission, 1989, The effects of xenobiotics on the structure and function of thyroid follicles and C-cells, *Toxicol. Pathol.* 17: 266.)

elevations in serum TSH levels may be related, in part, to the high thyroid iodine content (58% of molecular weight) interfering with the receptor-mediated response to TSH. The thyroid responsiveness to TSH is known to vary inversely with iodine content (17, 19). Thyroid glands of rats fed FD&C Red No. 3 would be exposed to an increased iodine primarily from sodium iodide contamination of the color and, to a lesser extent, from metabolism of the compound and release of iodide.

Induction of Hepatic Microsomal Enzymes. Hepatic microsomal enzymes play an important role in thyroid hormone economy because glucuronidation is the rate-limiting step in the biliary excretion of T_4 and sulfation by phenol sulfotransferase for the excretion of T_3. Long-term exposure of rats to a wide variety of different chemicals may induce these enzyme pathways and result in chronic stimulation of the thyroid by disrupting the hypothalamic–pituitary–thyroid axis (11). The resulting chronic stimulation of the thyroid by increased circulating levels of TSH often results in a greater risk of developing tumors derived from follicular cells in 2-yr or lifetime studies with these compounds in rats.

Xenobiotics that induce liver microsomal enzymes and disrupt thyroid function in rats include central nervous system–acting drugs (e.g., phenobarbital, benzodiazepines), calcium channel blockers (e.g., nicardipine, bepridil), steroids (spironolactone), retinoids, chlorinated hydrocarbons (e.g., chlordane, DDT, TCDD), and polyhalogenated biphenyls (PCB, PBB), among others. Most of the hepatic microsomal enzyme inducers have no apparent intrinsic carcinogenic activity and produce little or no mutagenicity or DNA damage. Their promoting effect on thyroid tumors usually is greater in rats than in mice, with males more often developing a higher incidence of tumors than females. In certain strains of mice, these compounds alter liver cell turnover and promote the development of hepatic tumors from spontaneously initiated hepatocytes.

Phenobarbital has been studied extensively as the prototype for hepatic microsomal inducers that increase a spectrum of cytochrome P-450 isoenzymes (22). McClain et al (21) reported that the activity of uridine diphosphate (UDP)–glucuronyltransferase, the rate-limiting enzyme in T_4 metabolism, is increased in purified hepatic microsomes of male rats when expressed as picomoles/min/mg microsomal protein (1.3-fold) or as total hepatic activity (3-fold). This resulted in a significantly higher cumulative (4-hr) biliary excretion of ^{125}I-T_4 and bile flow than in controls.

Phenobarbital-treated rats develop a characteristic pattern of changes in circulating thyroid hormone levels (21, 22). Plasma T_3 and T_4 are markedly decreased after 1 wk and remain decreased for 4 wk. By 8 wk, T_3 levels return to near normal due to compensation by the hypothalamic–pituitary–thyroid axis. Serum TSH values are elevated significantly throughout the first month but often decline after a new steady state is attained. Thyroid weights increase significantly after 2–4 wk of phenobarbital, reach a maximum increase of 40–50% by 8 wk, and remain elevated throughout the period of treatment.

McClain et al (22) in a series of experiments showed that supplemental administration of thyroxine (at doses that returned the plasma level of TSH to the normal range) blocked the thyroid tumor-promoting effects of phenobarbital and that the promoting effects were directly proportional to the level of plasma TSH in rats. The sustained increase in circulating TSH levels results initially in hypertrophy of follicular cells, followed by hyperplasia, and ultimately places the rat thyroid at greater risk to develop an increased incidence of benign tumors.

Phenobarbital has been reported to be a thyroid gland tumor promoter in a rat initiation–promotion model. Treatment with a nitrosamine followed by phenobarbital has been shown to increase serum TSH concentrations, thyroid gland weights, and the incidence of follicular cell tumors in the thyroid gland (21, 22). These effects could be decreased in a dose-related manner by simultaneous treatment with increasing doses of exogenous thyroxine.

McClain et al (21) demonstrated that rats treated with phenobarbital have a significantly higher cumulative biliary excretion of ^{125}I-thyroxine than controls. Most of the increase in biliary excretion was accounted for by an increase in T_4-glucuronide due to an increased metabolism of thyroxine in phenobarbital-treated rats. This is consistent with enzymatic activity measurements that result in increased hepatic T_4-UDP-glucuronyl transferase activity in phenobarbital-treated rats. Results from these experiments are consistent with the hypothesis that the promotion of thyroid tumors in rats was not a direct effect of phenobarbital on the thyroid gland but, rather, an indirect effect mediated by TSH secretion from the pituitary secondary to the hepatic microsomal enzyme-induced increase of T_4 excretion in the bile.

The activation of the thyroid gland during the treatment of rodents with substances that stimulate thyroxine catabolism is a well-known phenomenon and has been extensively investigated with phenobarbital and many other compounds (11). It occurs particularly with rodents, first because UDP-glucuronyl transferase can easily be induced in rodent species, and second because thyroxine metabolism takes place very rapidly in rats in the absence of thyroxine-binding globulin. In humans, a lowering of the circulating thyroxine level but no change in TSH and T_3 concentrations has been observed only with high doses of very powerful enzyme-inducing compounds such as rifampicin, both with and without antipyrine.

There is no convincing evidence that humans treated with drugs or exposed to chemicals that induce hepatic microsomal enzymes are at increased risk for the development of thyroid cancer (11). In a study of the effects of microsomal enzyme-inducing compounds on thyroid hormone metabolism in normal, healthy adults, phenobarbital (100 mg daily for 14 days) did not affect the serum T_4, T_3, or TSH levels (26). A decrease in serum T_4 levels was observed after treatment with either a combination of phenobarbital plus rifampicin or a combination of phenobarbital plus antipyrine; however, these treatments had no effect on serum T_3 or TSH levels (27). Epidemiologic studies of patients treated with therapeutic doses of phenobarbital have reported no increase in risk for the development of thyroid neoplasia (5–8, 14, 29, 32, 35). Highly sensitive assays for thyroid and pituitary

hormones are readily available clinically to monitor circulating hormone levels in patients who are exposed to chemicals that could potentially disrupt homeostasis of the pituitary–thyroid axis.

Likewise, there is no substantive evidence that humans treated with drugs or exposed to chemicals that induce hepatic microsomal enzymes are at increased risk for the development of liver cancer. This is best exemplified by the extensive epidemiologic information on the clinical use of phenobarbital. Phenobarbital has been used clinically as an anticonvulsant for more than 80 yr. Relatively high microsomal enzyme-inducing doses have been used chronically, sometimes for lifetime exposures, to control seizure activity in human beings. A study of more than 8,000 patients admitted to a Danish epilepsy center from 1933 to 1962 revealed no evidence for an increased incidence of hepatic tumors in phenobarbital-treated humans when patients receiving thorotrast, a known human liver carcinogen, were excluded (7). A more recent follow-up report on this patient population confirmed and extended this observation (29). The results of 2 other smaller studies (2,099 epileptics and 959 epileptics) also revealed no hepatic tumors in patients treated with phenobarbital (35).

Direct Effects of Xenobiotic Chemicals on the Thyroid Gland

Inhibition of Thyroid Hormone Synthesis: Blockage of Iodine Uptake by Thyroid. The initial step in the biosynthesis of thyroid hormones is the uptake of iodide from the circulation and transport against a gradient across follicular cells to the lumen of the follicle. A number of anions act as competitive inhibitors of iodide transport in the thyroid including perchlorate (ClO_4^-), thiocyanate (SCN^-), and pertechnetate (Fig. 10). Thiocyanate is a potent inhibitor of iodide transport and is a competitive substrate for the thyroid peroxidase but it does not appear to be concentrated in the thyroid. Blockage of the iodide trapping mechanism has a similar disruptive effect on the thyroid–pituitary axis to iodine deficiency. The blood levels of T_4 and T_3 decrease resulting in a compensatory increase in the secretion of TSH by the pituitary gland. The hypertrophy and hyperplasia of follicular cells following sustained exposure results in an increased thyroid weight and the development of goiter (Fig. 10).

Organification Defect Due to Inhibition of Thyroperoxidase. A wide variety of chemicals, drugs, and other xenobiotics affect the second step in thyroid hormone biosynthesis (Fig. 10). The step-wise binding of iodide to the tyrosyl residues in thyroglobulin requires oxidation of inorganic iodide (I^-) to molecular (reactive) iodine (I_2) by the thyroid peroxidase present in the luminal aspect (microvillar membranes) of follicular cells and adjacent colloid. Classes of chemicals that inhibit the organification of thyroglobulin include the (a) thionamides (such as thiourea, thiouracil, propylthiouracil, methimazole, carbimazole, and goitrin), (b) aniline derivatives and related compounds (e.g., sulfonamides, para-aminobenzoic acid, para-aminosalicylic acid, and amphenone), and (c) substituted phenols (such as resorcinol, phloroglucinol, and 2,4-dihydroxybenzoic acid) and miscellaneous inhibitors [e.g., aminotriazole, tricyanoaminopropene, antipyrine and its iodinated derivative (iodopyrine.)]

Many of these chemicals exert their action by inhibiting the thyroid peroxidase. This results in a disruption of both the iodination of tyrosyl residues in thyroglobulin and also the coupling reaction of iodotyrosines [e.g., monoiodotyrosine (MIT) and diiodotyrosine (DIT) to form iodothyronines (T_3 and T_4)]. In rats, propylthiouracil (PTU) has been shown to affect each step in thyroid hormone synthesis beyond iodide transport. The order of susceptibility to the inhibition by PTU is the coupling reaction (most susceptible), iodination of MIT to form DIT, and iodination of tyrosyl residues to form MIT (least susceptible). Thiourea differs from PTU and other thioamides in that it does not inhibit guaiacol oxidation (standard assay for peroxidase) and does not inactivate the thyroid peroxidase in the absence of iodine. Its ability to inhibit organic iodinations is due primarily to the reversible reduction of active I_2 to $2I^-$.

The goitrogenic effects of sulfonamides have been known for approximately 50 yr, since the reports of the action of sulfaguanidine on the rat thyroid. Sulfamethoxazole and trimethroprim exert a potent goitrogenic effect in rats, resulting in marked decreases in circulating T_3 and T_4, a substantial compensatory increase in TSH, and increased thyroid weights due to follicular cell hyperplasia. The dog also is a sensitive species to the effects of sulfonamides, with markedly decreased serum T_4 and T_3 levels, hyperplasia of thyrotrophic basophils, and increased thyroid weights.

By comparison, the thyroids of monkeys and humans are resistant to the development of changes that sulfonamides produce in rodents (rats and mice) and the dog. Rhesus monkeys treated for 52 wk with sulfamethoxazole (doses up to 300 mg/kg/day) with and without trimethroprim had no changes in thyroid weights and the thyroid histology was normal. Takayama et al (34) compared the effects of PTU and a goitrogenic sulfonamide (sulfamonomethoxine) on the activity of thyroid peroxidase in the rat and monkey using the guaiacol peroxidation assay. The concentration required for a 50% inhibition of the peroxidase enzyme was designated as the IC_{50}. When the IC_{50} for PTU was set at ×1 for rats, it took 50 times the concentration of PTU to produce a comparable inhibition in the monkey. Sulfamonomethoxine was almost as potent as PTU in inhibiting the peroxidase in rats with a factor of ×2.5. However, it required about 500 times the concentration of sulfonamide to inhibit the peroxidase in the monkey compared to the rat.

Studies such as these with sulfonamides demonstrate distinct species differences between rodents and primates in the response of the thyroid to chemical inhibition of hormone synthesis. It is not surprising that the sensitive species (e.g., rat, mouse, dog) are much more likely to develop follicular cell hyperplasia and thyroid nodules after long-term exposure to sulfonamides than the resistant species (e.g., subhuman primate, humans, guinea pig, chicken).

A contemporary example of a chemical acting as a thyroperoxidase inhibitor is sulfamethazine. This is a widely used antibacterial compound in food-producing

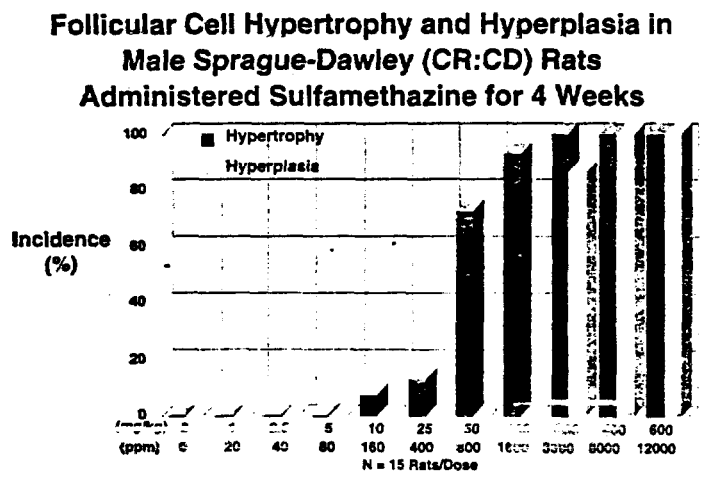

FIG. 11.—Nonlinear dose–response in morphologic changes in thyroid follicular cells in response to 10 dose levels of sulfamethazine administered in the feed to male Sprague-Dawley rats.

animals with a current permissible tissue residue level of 100 ppb. Recently completed carcinogenicity studies at NCTR reported a significant increase of thyroid tumors in male Fischer-344 rats administered the high dose (2,400 ppm) of sulfamethazine (20). The incidence of thyroid tumors was increased in both male and female $B_6C_3F_1$ mice after 2 yr in the high-dose (4,800 ppm) group but not in the lower dose groups. Quantitative risk assessment based on these new carcinogenicity findings, using low-dose linear extrapolation, yielded a 1×10^6 lifetime risk of 90 ppb in female rats and 40 ppb in male rats. A consideration of the ratio of intact drug to metabolites further reduced the tissue residue level to 0.4 ppb, which would be unachievable in practice (20).

In collaboration with Dr. M. McClain (Hoffman La Roche, Nutley, NJ) and others, a number of mechanistic studies were performed with the objective of developing a database that would support the hypothesis that the thyroid tumors observed in rats and mice from chronic studies were secondary to hormonal imbalances following the administration of high doses of sulfamethazine. In a 4-wk mechanistic study, the effects of 10 dose levels (0 > 12,000 ppm) of sulfamethazine, spanning the range that induced thyroid tumors in rodents, were evaluated on thyroid hormone economy in Sprague-Dawley rats. There was a characteristic log dose–response relationship in all parameters of thyroid function evaluated. There were no significant changes at the 6 lower doses (20 > 800 ppm) of sulfamethazine, followed by sharp relatively linear changes at the 4 higher dose levels (1,600 > 12,000 ppm) in percentage of decrease of serum T_3 and T_4, increase in serum TSH, and increase in thyroid weight. A similar, nonlinear dose–response was present in the morphologic changes of thyroid follicular cells following the feeding of varying levels of sulfamethazine. Follicular cell hypertrophy was observed at lower doses of sulfamethazine than hyperplasia, which was increased only at dose levels of 3,300 ppm and above (Fig. 11). Other mode-of-action studies have demonstrated sulfamethazine to be a potent inhibitor of thyroperoxidase in rodents with an IC_{50} of 1.2×10^{-6} M. The morphologic effects on the thyroid were reversible after withdrawal of compound, and supplemental T_4 in the diet inhibited the development of the functional and morphologic changes in thyroid follicular cells (20). Hypophysectomized rats (with no TSH) administered sulfamethazine did not develop morphologic changes in the thyroid. Sulfamethazine did not increase thyroid cell proliferation *in vitro* in the absence of TSH, and there was no effect on thyroid structure/function in cynomolgus monkeys administered sulfamethazine. Nonhuman primates and humans are known to be more resistant than rodents to the inhibition of thyroperoxidase.

Inhibition of Thyroid Hormone Secretion: Blockage of Thyroid Hormone Release by Excess Iodide or Lithium. Relatively few chemicals selectively inhibit the secretion of thyroid hormone from the thyroid gland (Fig. 7). An excess of iodine has been known for years to inhibit secretion of thyroid hormone and occasionally can result in goiter and hypothyroidism in animals and human patients. High doses of iodide have been used therapeutically in the treatment of patients with Grave's disease and hyperthyroidism to lower circulating levels of thyroid hormones. Several mechanisms have been suggested for this effect of high iodide levels on the thyroid hormone secretion including a decrease in lysosomal protease activity (human glands), inhibition of colloid droplet formation (mice and rats), and inhibition of TSH-mediated increase in cyclic adenosine monophosphate (cAMP) (dog thyroid slices). In my laboratory, rats fed an iodide-excess diet had a hypertrophy of the cytoplasmic area of follicular cells with an accumulation of numerous colloid droplets and lysosomal bodies. However, there was limited evidence ultrastructurally of fusion of the membranes of these organelles and degradation of the colloid necessary for the release of T_4 and T_3 from the thyroglobulin (9). Circulating levels of T_4, T_3, and rT_3 all are decreased by an iodide excess.

Lithium also has been reported to have a striking inhibitory effect on thyroid hormone release (Fig. 10). The widespread use of lithium carbonate in the treatment of manic states occasionally results in the development of goiter with either euthyroidism or occasionally hypothyroidism in human patients. Lithium inhibits colloid droplet formation stimulated by cAMP *in vitro* and inhibits the release of thyroid hormones.

Xenobiotic-Induced Thyroid Pigmentation or Alterations in Colloid. The antibiotic minocycline produces a striking black discoloration of the thyroid lobes in laboratory animals and humans with the formation of brown pigment granules within follicular cells. The pigment granules stain similarly to melanin and are best visualized on thyroid sections stained with the Fontana-Masson procedure. Electron-dense material first accumulates in lysosome-like granules and in the rough endoplasmic reticulum. The pigment appears to be a metabolic derivative of minocycline, and the administration of the antibiotic at high dose to rats for extended periods may result in a disruption of thyroid function and the development of goiter. The release of T_4 from perfused thyroids of minocycline-treated rats was significantly decreased, but the follicular cells retained the ability to phagocytose colloid

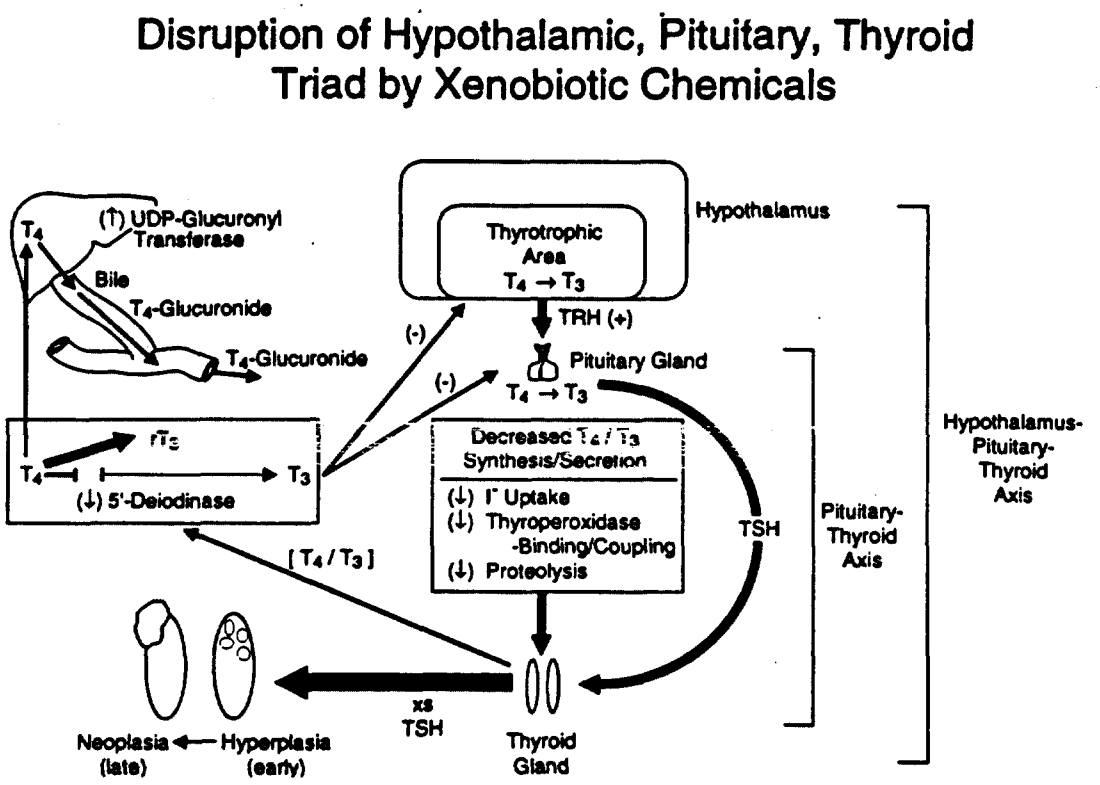

FIG. 12.—Multiple sites of disruption of the hypothalamic–pituitary–thyroid triad by xenobiotic chemicals. Chemicals can exert direct effects by disrupting thyroid hormone synthesis or secretion and indirectly influence the thyroid through an inhibition of 5'-deiodinase or by inducing hepatic microsomal enzymes (e.g., T_4-UDP-glucuronyl transferase). All of these mechanisms can lower circulating levels of thyroid hormones (T_4 and T_3), resulting in a release from negative feedback inhibition and increased secretion of TSH by the pituitary gland. The chronic hypersecretion of TSH predisposes the sensitive rodent thyroid gland to develop an increased incidence of focal hyperplastic and neoplastic (adenomas) lesions by a secondary (epigenetic) mechanism due to hormonal imbalances.

in response to TSH and had numerous colloid droplets in their cytoplasm.

Other xenobiotics [or metabolite(s)] selectively localize in the thyroid colloid of rodents, resulting in abnormal clumping and increased basophilia to the colloid. Brown to black pigment granules may be present in follicular cells, colloid, and macrophages in the interthyroidal tissues resulting in a macroscopic darkening of both thyroid lobes. The physiochemically altered colloid in the lumen of thyroid follicles appears to be less able than normal colloid of either reacting with organic iodine in a stepwise manner to result in the orderly synthesis of iodothyronines or being phagocytized by follicular cells and enzymatically processed to release active thyroid hormones into the circulation. Serum T_4 and T_3 are decreased, serum TSH levels are increased by an expanded population of pituitary thyrotrophs, and thyroid follicular cells undergo hypertrophy and hyperplasia. As would be expected, the incidence of thyroid follicular cell tumors in 2-yr carcinogenicity studies is significantly increased at the higher dose levels, usually with a greater effect in males than in females. Autoradiographic studies often demonstrate tritiated material to be preferentially localized in the colloid and not within follicular cells. Tissue distribution studies with ^{14}C-labeled compound may reveal preferential uptake and persistence in the thyroid gland compared to other tissues. However, thyroperoxidase activity is normal, and the thyroid's ability to take up radioactive iodine often is increased compared to controls in response to the greater circulating levels of TSH. Similar thyroid changes and/or functional alterations usually do not occur in dogs, monkeys, or humans.

SECONDARY MECHANISMS OF THYROID ONCOGENESIS

Understanding the mechanism of action of xenobiotics on the thyroid gland provides a more rational basis to extrapolate findings from long-term rodent studies to safety assessment of a particular compound for humans. Many chemicals and drugs disrupt one or more steps in the synthesis and secretion of thyroid hormones, resulting in subnormal levels of T_4 and T_3 associated with a compensatory increased secretion of pituitary TSH (Fig. 12). When tested in highly sensitive species, such as rats and mice, these compounds result early in follicular cell hypertrophy/hyperplasia and increased thyroid weights and in long-term studies an increased incidence of thyroid tumors by a secondary (indirect) mechanism.

In the secondary mechanism of thyroid oncogenesis in rodents, the specific xenobiotic chemical or physiologic perturbation evokes another stimulus (e.g., chronic hypersecretion of TSH) that promotes the development of nodular proliferative lesions (initially hypertrophy, followed by hyperplasia, subsequently adenomas, infre-

quently carcinomas) derived from follicular cells. Thresholds for a no-effect on the thyroid gland can be established by determining the dose of xenobiotic that fails to elicit an elevation in the circulating level of TSH. Compounds acting by this indirect (secondary) mechanism with hormonal imbalance usually have little or no evidence for mutagenicity or for producing DNA damage.

In human patients with markedly altered changes in thyroid function and elevated TSH levels, as in areas with a high incidence of endemic goiter due to iodine deficiency, there is little if any increase in the incidence of thyroid cancer (11, 13). The relative resistance to the development of thyroid cancer in humans with elevated plasma TSH levels is in marked contrast to the response of the thyroid gland to chronic TSH stimulation in rats and mice. The human thyroid is much less sensitive to this pathogenetic phenomenon than rodents (21).

Human patients with congenital defects in thyroid hormone synthesis (dyshormonogenetic goiter) and markedly increased circulating TSH levels have been reported to have an increased incidence of thyroid carcinomas (10, 23). Likewise, thyrotoxic patients with Grave's disease where follicular cells are autonomously stimulated by an immunoglobulin (long-acting thyroid stimulator, or LATS) also appear to be at greater risk to develop thyroid tumors (4, 31). In summary, the literature suggests that prolonged stimulation of the human thyroid by TSH will induce neoplasia only in exceptional circumstances and possibly acting together with some other metabolic or immunologic abnormality (11).

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